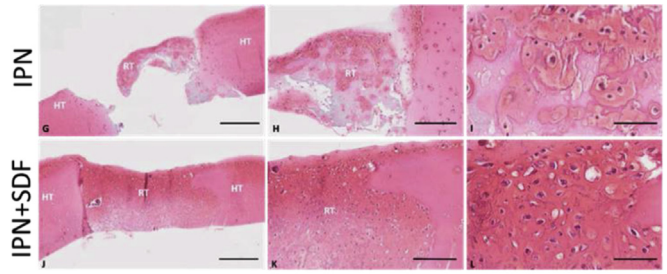
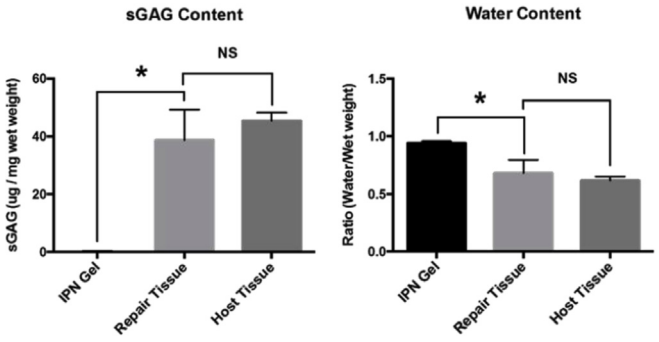
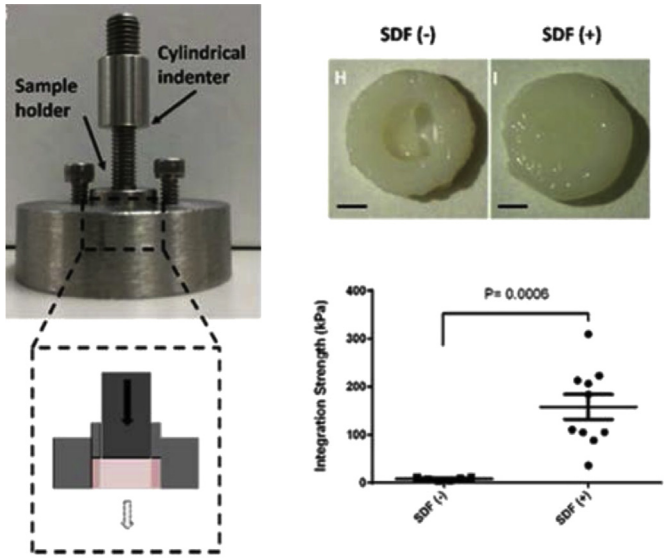
cartilage (Figure 2). A customized cartilage fixation device was used to measure integration strength (Figure 3). The test proceeded through the full depth of the tissue, and the maximum force recorded was divided by the area of integration to determine the integration strength. Good adhesion with surrounding cartilage was confirmed by push-out tests, which gave interfacial strength values of 158 +/- 82 kPa for SDF-1-treated defects (Figure 3) All 4 material property measurements (maximum and equilibrium stress, Young's modulus, and maximum force) were similar in neocartilage than in native cartilage (Table 1).

Conclusions: We have developed a cartilage repair strategy that exploits the regenerative potential of endogenous chondrogenic progenitor cells. The matrix formed by these cells is similar in composition to native cartilage and strongly adheres to surrounding tissues. In addition, neocartilage is functional, and has mechanical properties comparable with native cartilage. Future studies are needed to for optimization when translated to in vivo system.







Summary of mechanical properties

Sample	Maximum stress kPa	Equilibrium stress kPa	Young's Modulus kPa	Maximum force N
Native Cartilage	152.4	16.4	1228.1	1.9
stdev	11.1	1.9	158.3	0.3
Neo-Cartilage	98.7	5.9	746.7	1.2
stdev	22.7	0.9	82.3	0.1
Native/ Neo	1.5	2.7	1.6	1.5

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AN AGGRECAN 32-MER FRAGMENT GENERATED IN OSTEOARTHRITIS DIRECTLY EXCITES NOCICEPTIVE NEURONS

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Purpose: An early event in the pathogenesis of osteoarthritis (OA) is cleavage of aggrecan in the interglobular domain (E373-374A) by ADAMTS-4/5. Further cleavage by MMPs (N341-342F) releases a 32-amino-acid fragment (32-mer). We have previously shown that this 32-mer fragment is a TLR2-dependent damage-associated molecular pattern (DAMP) molecule in chondrocytes, synovial fibroblasts and in peritoneal macrophages. Here, we hypothesized that the 32-mer aggrecan fragment may activate nociceptors, thus contributing to OA joint pain. We investigated the effects of the 32-mer on primary cultures of dorsal root ganglia (DRG) cells and in DRG explants.

Methods: Knee-innervating DRG cells (L3-L5) were acutely isolated and pooled from 4 adult C57BL/6 wild-type, Tlr4-null, or Tlr2-null mice and cultured in neurogenic medium. Direct interactions of the 32-mer with DRG neurons were monitored by examining its effects on intracellular calcium (Ca)i or the expression of the pro-algesic chemokine MCP-1. For Ca mobilization assays, cells were loaded with Fura-2, and responses to synthetic mouse 32-mer (FFGVGGEDDITIQTVTWPDLELPLPRNVTEGE) or to scrambled peptide were recorded in >100 neurons. For MCP-1 stimulation assays, cells were treated overnight with 32-mer (0.3-30 μM) or with scrambled peptide. Supernatants were collected for ELISA of MCP-1. MCP-1 was chosen as a marker of activation because we have previously shown that this is an important mediator of pain in experimental OA.

For ex vivo imaging assays, intact DRG explants (L4 or L5) were isolated from Pirt-GCaMP3 mice. These mice express the fluorescent calcium indicator, GCaMP3, in ~90% of all sensory DRG neurons, and not in other peripheral or central tissues, through the Pirt promoter. Explants were placed in a perfusion chamber within artificial cerebrospinal fluid and imaged using a spinning disk confocal microscope at the 488 nm wavelength. Explants were stimulated by injecting 10 μL of solution containing 1 mM 32-mer into a continuously running perfusion chamber with a volume of 1 mL. Image analysis was performed using ImageJ to determine change in fluorescence intensity with time.

Results: Cultured DRG neurons rapidly responded to 32-mer peptide, but not scrambled control peptide, as indicated by increased (Ca)i in 19% of neurons. This suggests that DRG neurons express excitatory receptors for this protein fragment. The majority of 32-mer responses were seen in small-to-medium-diameter neurons (nociceptors) that were also responsive to capsaicin, which demonstrates that TRPV1-expressing nociceptors are capable of responding to 32-mer. In order to show that 32-mer responses are not an artifact of cell culture, calcium imaging was also performed using intact DRG from Pirt-GCaMP3 mice. Within DRG explants, 7% of neurons were able to respond to 32-mer peptide, while scrambled peptide elicited no responses.

Overnight stimulation of cultured DRG cells with either 3 or 30 μM 32-mer peptide resulted in significant upregulation in MCP-1 protein production compared to unstimulated cells (3.1-fold (3 μM) and 3.5-fold (30 μM), $p<0.001$). The highest concentration of scrambled peptide (30 μM) did not induce MCP-1 production (0.8-fold, $p=0.09$ vs unstimulated).

In order to investigate which receptor may be mediating the upregulation of MCP-1, DRG cells were cultured from Tlr2 null or Tlr4 null mice. Stimulation with 32-mer peptide (3 μM) produced increased MCP-1 in Tlr4 null DRG cells compared to unstimulated cells (3.3-fold, $p<0.01$),

but not in Tlr2 null cells (0.9-fold, p=0.5), suggesting that these effects are mediated through TLR2. DRG cells also respond to a synthetic TLR2 ligand (Pam3CSK4, 10 μM, 7.7-fold, p<0.001), providing additional evidence that TLR2 signaling can lead to MCP-1 upregulation.
Conclusions: These studies suggest that, through TLR2, nociceptors have the ability to respond to a specific 32-mer cleavage product of aggrecan. This pathway may play a role in the development of osteoarthritis-associated pain.

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GREMLIN1 INDUCED BY EXCESSIVE MECHANICAL STRESS LOADING ENHANCES CARTILAGE DEGRADATION

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Purpose: Excessive mechanical stress loading on articular cartilage is one of the major factors of osteoarthritis (OA); however, the molecular mechanisms of cartilage degradation by such loading remain unclear. The present study therefore analyzed gene expression profiles of articular chondrocytes under mechanical stress loading and investigated functions of the candidate gene.
Methods: We applied an uni-axial cyclic cell stretch (0.5 Hz, 10% stretch, for 30 min) to primary articular chondrocytes obtained from mouse knee joint by STB-140 (STREX), and performed cDNA microarray analysis using the samples before and after the loading. We also applied excessive hydrostatic pressure (0.1 Hz, 20 MPa, for 30 min) to 3-week-old mouse femoral heads by a custom-made device, and compared the results in both systems. The gene expressions were confirmed by real-time RT-PCR and immunohistochemistry. The catabolic ability was assessed by measuring the concentration of aggrecan released from cultured 3-week-old mouse femoral heads using the dimethylmethylene blue dye-binding assay. We used mouse primary articular chondrocytes and mouse chondrogenic cell line ATDC5 for in vitro functional analyses. We further examined in vivo effects of a candidate gene using a surgical OA model of mouse knee joint.
Results: Microarray analyses revealed that 2,076 genes were increased more than twice by the cell stretch. Among them, we focused on Gremlin1 (Grem1), which is one of the most highly expressed genes. Grem1 is known to be a secretory protein that regulates limb development, although its function in articular cartilage is unknown. Immunocytochemistry confirmed that Grem1 protein was increased in cytoplasm of articular chondrocytes by the cell stretch. Grem1 mRNA expression was also increased in mouse femoral heads under excessive cyclic hydrostatic pressure. Immunofluorescence further revealed that Grem1 protein was increased on the cell surface of articular chondrocytes during OA development of the mouse model. In primary culture of mouse articular chondrocytes, recombinant human (rh) GREM1 treatment or adenoviral overexpression of Grem1 induced the expressions of catabolic factors including Mmp13 and Adamts5, and suppressed those of anabolic factors including Col2a1, Aggrecan and Sox9. Similar results were observed in ATDC5 by lentiviral transduction of Grem1. Microarray analyses showed that other various catabolic and inflammatory factors, such as Mmp3, Mmp8, Il1b (IL-1β), and Il6 (IL-6), were significantly upregulated by the rhGREM1 treatment in mouse articular chondrocytes. In the organ culture of mouse femoral heads, rhGREM1 treatment increased aggrecan release into the medium in a dose-dependent manner. In the mouse OA model, intra-articular injection of rhGREM1 exacerbated cartilage degradation. In contrast, intra-articular injection of Grem1 neutralizing antibody inhibited OA development.
We further found that Grem1 enhanced the NF-κB signaling activation by luciferase assay. Notably, in the organ culture of femoral heads, the increased aggrecan release by the rhGREM1 treatment was completely cancelled by gene deletion of p65/Rela, or an IKK inhibitor (BMS-345541) treatment, indicating that the catabolic effects of Grem1 were mediated by the NF-κB signaling pathway.
Conclusions: Grem1 is induced by excessive mechanical stress loading, and exerts catabolic effects on articular cartilage through the NF-κB signaling pathway. Grem1 may be a novel therapeutic target of OA.

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INCIDENCE, PREVALENCE, NATURAL COURSE AND PROGNOSIS OF PATELLOFEMORAL OSTEOARTHRITIS; DATA OF COHORT HIP AND COHORT KNEE STUDY

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Purpose: Most research in osteoarthritis (OA) has focused on the tibiofemoral (TF)-joint, although it is proposed that signs of patellofemoral (PFOA), opposed to TFOA, are associated with pain symptoms and disability. Additionally, it is suggests that OA in the knee starts in the PF joint and progresses to the TF joint. Therefore, this study addresses the following aims: 1. Determine the prevalence and incidence of PFOA compared to TFOA in patients with a recent onset of knee pain 2. Describe the natural course of both PFOA and TFOA after 2 and 5 years follow-up and 3. Identify if patients with PFOA have a different phenotype compared to patients with TFOA, or patients with combined PFOA and TFOA.
Methods: For this study data of the Cohort Hip and Cohort Knee study (CHECK) were used (n= 1002). Patients with early OA symptoms of the knee were selected. All patients completed a questionnaire and underwent physical examination at baseline and after 2 and 5 years follow-up. At each time-point, three radiographs of the knee were obtained (Anterior-Posterior, skyline and lateral). Following these radiographs, all patients were classified into four different groups: isolated TFOA, isolated PFOA, combined (TF and PF) OA (COA) or no radiographic OA. Only the knee with most severe symptoms per patient was analyzed. Multivariate backward stepwise binary logistic regression (p<.05) was used to identify if patient characteristics and characteristics from physical examination were associated with a specific group of OA patients. The following variables were included in the regression analyses: gender, age, body mass index (BMI), baseline pain, bony tenderness during palpation, crepitus in the knee duration flexion, degrees of knee flexion, patellar grinding test.
Results: The total cohort comprised 845 patients (80% females) with a mean age of 55.3 (5.18) years. The prevalence and incidence of the different types of OA are presented in Table 1. At baseline the majority of patients had no radiographic signs of OA. The natural course of the different patient subgroups is presented in Figure 1. Twenty-seven out of the 83 patients who had PFOA at baseline remained to have PFOA at 5 years follow-up; 56 developed COA after two to five yours follow-up.
Patients with PFOA at baseline were more likely to have a higher BMI (OR (95%CI) 1.09 (1.03;1.16), higher age (1.07 (1.01;1.13), positive patellar grinding test (1.92 (1.12;3.29) and palpable bony tenderness of the knee (2.90 (1.09;7.76) compared to patients without radiographic OA at baseline. After two years, patients with PFOA did not differ from patients with TFOA, however patients with PFOA, had lower BMI scores at baseline compared to patients with COA (OR (95% CI) 0.90 (0.82;0.99).However no differences were seen after five year follow-up. Patients with signs of PFOA at baseline who did not develop COA after 5 years had significantly higher active knee flexion range of motion (ROM), compared to patients who had PFOA at baseline and developed COA at follow-up (OR (95%CI) 1.10 (1.01;1.20).
Conclusions: After 5 years follow-up 33% of the primary care patients with knee pain had developed OA (PFOA, TFOA or COA). About one third of the patients with PFOA still have PFOA after 5 years and these patients distinguish in a high active knee flexion ROM. Patients with PFOA at baseline significantly differ from patients without any signs of radiographic OA at baseline. However phenotypes of patients with PFOA do not seem to differ from patients with TFOA or COA.

Table 1
Prevalence of OA divided by subgroups of OA

	PFOA	TFOA	COA	No radiographic OA	Total
Baseline	83	0	0	629	712
2 year	51	53	68	547	719
5 year	76	73	86	468	703